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SYMPOSIUM

Move That Fatty Acid: Fuel Selection and Transport in Migratory Birds and Bats

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Synopsis The metaphor of marathon running is inadequate to fully capture the magnitude of long-distance migratory flight of birds. In some respects a journey to the moon seems more appropriate. Birds have no access to supplementary water or nutrition during a multi-day flight, and they must carefully budget their body fat and protein stores to provide both fuel and life support. Fatty acid transport is crucial to successful non-stop migratory flight in birds. Although fat is the most energy-dense metabolic fuel, the insolubility of its component fatty acids makes them difficult to transport to working muscles fast enough to support the highly aerobic exercise required to fly. Recent evidence indicates that migratory birds compensate for this by expressing large amounts of fatty acid transport proteins on the membranes of the muscles (FAT/CD36 and FABPpm) and in the cytosol (H-FABP). Through endogenous mechanisms and/or diet, migratory birds may alter the fatty acid composition of the fat stores and muscle membranes to improve endurance during flight. Fatty acid chain length, degree of unsaturation, and placement of double bonds can affect the rate of mobilization of fatty acids from adipose tissue, utilization of fatty acids by muscles, and whole-animal performance. However, there is great uncertainty about how important fatty acid composition is to the success of migration or whether particular types of fatty acids (e.g., omega-3 or omega-6) are most beneficial. Migratory bats provide an interesting example of evolutionary convergence with birds, which may provide evidence for the generality of the bird model to the evolution of migration by flight in vertebrates. Yet only recently have attempts been made to study bat migration physiology. Many aspects of their fuel metabolism are predicted to be more similar to those of migrant birds than to those of non-flying mammals. Bats may be distinct from most birds in their potential to conserve energy by using torpor between flights, and in the behavioral and physiological trade-offs they may make between migration and reproduction, which often overlap.

Introduction

The title of this contribution is meant to bring attention to the fact that the ability of non-soaring birds to undertake long-distance migratory flights is critically dependent on a very high capacity for fatty acid transport, particularly in flight muscle. Key alterations to protein-mediated fatty acid transport mechanisms are necessary to allow fatty acids to be used to fuel the intense exercise required for flying, and these make migratory birds (and possibly bats) relatively unique among vertebrates.

My own journey towards this understanding began when I started to study the migration physiology of shorebirds. Papers by Weber (1988, 1992), Butler (1991), and by Taylor's and Weibel's collaborative research group (*Journal of Experimental Biology* Special Issue 1996, Volume 199) made it apparent that birds must be very different from mammals in their patterns of fuel selection during exercise, especially during migration. A fortuitous interaction with Norbert Haunerland, who studied cytosolic fatty acid binding protein in desert locusts (*Schistocerca gregaria*) (Haunerland 1994), opened my eyes to the potential for muscle fatty acid transporters to be important for migratory flight. Early studies of the biochemistry of the flight muscles of migratory birds generally focused on the capacity for

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oxidation of substrates, not the importance of fuel (fatty acid) transport (e.g., Marsh 1981, Lundgren and Kiessling 1985, Driedzic et al. 1993). It is now recognized that, indeed, dramatic seasonal upregulation of fatty acid transporters in flight muscle is a crucial aspect of bird migration (Guglielmo et al. 1998, Pelsers et al. 1999, Guglielmo et al. 2002a, McFarlan et al. 2009).

Stories like the annual migration of the bar-tailed godwit (*Limosa lapponica baueri*) continually inspire study of the physiology and ecology of migratory birds. Recent satellite telemetry shows that this species can fly for as long as nine days without food or water, travelling as far as 11,000 km over the open ocean between breeding grounds in Alaska and wintering grounds in New Zealand (Gill et al. 2009). This is a phenomenal feat of endurance, navigation, and sleep deprivation, and we must wonder how they possibly budget all of the fuel (fat and protein) and water for such a journey. Usually, we tend to compare this with some kind of extreme marathon running, but I no longer believe this is an adequate metaphor. Human marathoners have access to water, sugar and electrolytes during their run, and they have a completely different fuel strategy based on carbohydrates (hence the practice of "carbo-loading").

Lately, I have been thinking that a multi-day migratory flight is more like a moon shot than a marathon. A trip to the moon takes only ~5 days one way, the passengers are travelling through a hostile, potentially deadly environment, and may be going to a place they have never experienced before. Budgeting of fuel and life support are critical. Birds, however, are both the vehicle, as well as the precious living cargo, and they have to manage fuel and life support systems that are intimately and inextricably linked by metabolism. Decisions related to maintaining physiological homeostasis will affect the use of fuel and vice versa. Birds must have everything on board before departure, and miscalculation or budgeting errors can result in death. The Apollo 11 lunar module landed with <30 s of fuel remaining to control its descent. For birds, as with humans, there must be very strong selection to get everything right and avoid disaster.

One might consider the adipose fat stored outside of the muscle cells as the fuel for a main thruster of our bird-rocket and protein as analogous to the fuel for small maneuvering jets; both are critical but for very different reasons. Carbohydrate (glycogen) stores are generally considered to be unimportant as fuel for a migratory flight of any significant distance. Fat is the clear choice as a metabolic fuel for migration because it is light and energy-dense. On a wet-mass basis adipose fat has about eight to ten times more energy than do either carbohydrate or protein (Jenni and Jenni-Eiermann 1998). Unfortunately, fat alone cannot meet all of the metabolic needs during a flight because the end products of fatty acid catabolism (e.g., acetyl-coenzyme A) cannot be used to synthesize new glucose or various other key metabolites required in the citric acid cycle (Jenni and Jenni-Eiermann 1998). However, amino acids, stored as functional proteins in muscles and organs, are abundant and can be used for gluconeogenesis and for a variety of other essential metabolic pathways. Catabolism of wet protein also yields about five times more water per unit of energy than does fat (Jenni and Jenni-Eiermann 1998), so that its use could be important under dehydrating conditions.

There is ample empirical evidence that a mixture of fat and protein fuels migratory flight in birds (Lindström and Piersma 1993, Jenni and Jenni-Eiermann 1998, McWilliams et al. 2004). Large extra-muscular fat deposits are obvious under the skin and in the abdominal cavity of migrants, and significant deposition of lean mass, mainly protein, has also been recorded (Lindström and Piersma 1993, Guglielmo and Williams 2003). In general, the available data suggest that ~90% of the energy for migratory flight comes from fat and the remaining 10% from protein (Jenni and Jenni-Eiermann 1998). Although we know this to be true, it is difficult to reconcile with what is known about exercise and fuel selection from the extensive literature on mammals, and therefore we must seek to explain the physiological and biochemical mechanisms birds use to fly while burning fatty acids. Below I discuss how birds are able to use fat for high-intensity exercise, and then comment on: (1) how fatty acid composition of fat stores and muscle membranes may affect performance during flight, and (2) possible parallels with migratory bats.

Fat and exercise

Running mammals have very poor ability to use fat as a fuel for high-intensity aerobic exercise (Fig. 1) (Roberts et al. 1996, McClelland 2004). Across a wide range of species the contribution of fat oxidation to total energy demand is high at rest and during low-intensity exercise. However, as the intensity of exercise increases the relative importance of fat declines precipitously, such that near the maximum rate of oxygen consumption ($\dot{V}O_2$ max), fat contributes only 10–20% of the fuel demanded. Carbohydrate oxidation, mainly from intramuscular

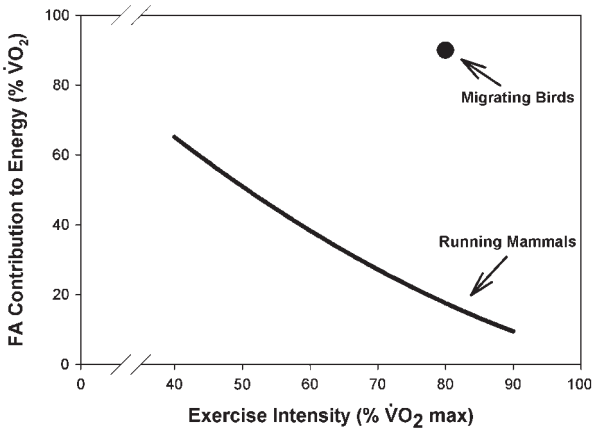

Fig. 1 The contribution of fatty acid oxidation to total energy demand as a function of relative exercise intensity for a variety of running mammals (dogs, goats, rats, and humans). A hypothetical data point for migratory birds is plotted to illustrate that unlike mammals, birds can exercise at a high intensity and use fatty acids as fuel. Modified from Fig. 2 in McClelland (2004).

glycogen, makes up most of the deficit, with protein contributing such a small fraction that is often ignored (usually to simplify calculations of indirect calorimetry). Furthermore, studies using isotopic tracers generally show that only about half of the fatty acids oxidized by mammals during running originate from adipose stores outside of the muscles (Weber et al. 1996). To use only fat depots for energy a mammal must exercise at very low intensity and walk (Roberts et al. 1996). The problem for birds is that they never exercise at a low intensity during flapping flight. There is no equivalent to "walking" because the power curve is U-shaped (flying slowly actually requires more power than flying at an intermediate speed), and the bottom of the curve (minimum power speed) is thought to be equivalent to about twice the $\dot{V}O_2$ max of a running mammal of similar body size (Butler and Woakes 1990). True $\dot{V}O_2$ max has never been measured in a flying bird, and it may not be possible to do so using the graduated exercise test used for running mammals. It has been estimated that migrating birds may exercise at 70–90% $\dot{V}O_2$ max (Guglielmo et al. 2002a). With 90% of their energy coming from adipose fatty acids birds would be far off the fuel selection curve typical for mammals (Fig. 1).

Fat is more difficult to use as a fuel than are carbohydrates or protein because fatty acids are extremely insoluble in water. Unlike glucose or amino acids, fatty acids cannot dissolve in the cytosol or plasma, and so they require protein carriers at nearly every step of their transport from fat stores, through the circulation, and to sites of oxidation inside muscles. A simple hypothesis is that mammals cannot use fatty acids at very high rates simply because they do not invest enough in the production of fatty acid carriers and transporters, whereas migratory birds are under strong selection to do so.

Vock et al. (1996) suggested that the major bottlenecks for the flux of fatty acids exist in the circulation and particularly at the sarcolemmal membrane, and so these are obvious targets for upregulation in birds, especially in migratory species and during migratory seasons. Circulatory transport of fatty acids appears to be accelerated in some migrant passerine birds by a lipoprotein-mediated pathway (Jenni-Eiermann and Jenni 1992), a possibility that deserves further experimental study. Other studies have focused on fatty acid transporters in flight muscle. The heart-type fatty acid binding protein (H-FABP) is a cytosolic carrier of fatty acids whose abundance is correlated with cellular capacity for oxidation of fatty acids (Haunerland 1994). The flight muscles of migratory western sandpipers (*Calidris mauri*) have very high concentrations of H-FABP (~10-fold greater than typical mammalian muscles), and H-FABP is 70% more abundant during migratory seasons than during winter residency (Guglielmo et al. 1998, 2002a). Barnacle geese (*Branta leucopsis*) show a similar change in H-FABP expression during migration (Pelsers et al. 1999). Like many birds, western sandpipers have very red, myoglobin-rich flight muscles, reflecting their high aerobic capacity. Visual inspection of SDS–PAGE gels makes it readily apparent that H-FABP is nearly as abundant as myoglobin in the flight muscle of sandpipers, and I believe if H-FABP was as darkly colored as myoglobin its importance to migration may have been recognized long ago. Most importantly, the seasonal pattern of expression indicates that H-FABP is particularly important during migration when the demand for oxidation of extramuscular fatty acids is most acute. Otherwise, why should birds down-regulate this gene during the winter, unless it is costly to maintain such high fatty acid transport machinery when it is unnecessary?

The cytosolic H-FABP promotes uptake of fatty acids into muscle cells by accepting fatty acids from the membrane, but the sarcolemma itself can also act as a barrier to entry. Beginning in the 1990s, it was recognized that a substantial proportion (80%) of membrane transport may be mediated by proteins such as fatty acid translocase (FAT/CD36) and plasma-membrane fatty acid binding protein (FABPpm; McArthur et al. 1999). Recently, McFarlan et al. (2009) showed that white-throated sparrows (*Zonotrichia albicollis*) express FAT/CD36

and FABPpm in flight muscle, and that these genes, as well as the H-FABP gene, are strongly up-regulated during migratory seasons. Thus, there is good evidence from passerine and non-passerine birds that at least three muscle fatty acid transport proteins are of major importance to the fuel metabolism of migrating birds.

The next step will be to understand how fatty acid transport proteins are controlled by endogenous and exogenous factors, such as photoperiod and exercise, and to elucidate the endocrine mechanisms involved. A recent experiment with captive white-throated sparrows, showed that expression of FAT/CD36 and H-FABP (but not FABPpm) was increased by a shift in photoperiod from winter to spring-migratory conditions (Zajac D, Cerasale D, Guglielmo CG, unpublished data). Clearly, the up-regulation of muscle fatty acid transporters during migration can be dramatic and supports their importance for endurance flight. Continued study to understand the regulation and functional significance of these proteins, as well as other steps in fatty acid transport, should prove very rewarding.

Fatty acid composition and migratory performance

Whereas there is no question that fat is indispensible as a fuel for migratory flight, there is debate about how the fatty acid composition of fat stores and/or of muscle membranes may affect metabolism and migratory performance of birds. Price (2010) extensively reviews this issue, and readers are directed to that paper for a full treatment of the evidence and for development of a theoretical framework. The physico-chemical properties of fatty acids, and their roles in metabolism, are affected by length of the carbon chain (generally 12–24) and degree of unsaturation (0–6 carbon–carbon double bonds). Shorter, more unsaturated fatty acids are more hydrophilic and have lower melting points, potentially making them more rapidly metabolized than are longer and/or saturated fatty acids. Polyunsaturated fatty acids (PUFAs) have two or more double bonds. The majority of PUFAs in animals have the first double bond either three (n3 or omega-3) or six (n6 or omega-6) carbons from the methyl end of the molecule. Birds can synthesize saturated and monounsaturated fatty acids, but do not have the enzymatic capacity to insert double bonds at the n3 or n6 positions (Stevens 1996). Therefore, most PUFAs must be derived from dietary sources, although dietary precursors such as 18:2n6 or 18:3n3 can be elongated or further desaturated to other PUFAs such as 20:4n6 or 20:5n3 (Stevens 1996). The resultant fatty acid composition of triglycerides in fat stores and in phospholipids in membranes of birds is influenced by dietary composition, *de novo* synthesis, and endogenous regulation (Price 2010).

Birds have sometimes been observed to change their fatty acid composition coincident with migration, but the patterns of variation are inconsistent (Pierce and McWilliams 2005). A question of interest has been whether greater unsaturation or increases of n3 or n6 PUFAs may improve flight performance. More unsaturated fatty acids may be better as fuel because they are preferentially mobilized from bird adipocytes (Price et al. 2008), and more rapidly oxidized by bird flight muscles (Price ER, Staples JF, Milligan CM, Guglielmo CG, unpublished data). Increased unsaturation (especially PUFAs) of muscle plasma and mitochondrial membranes could affect the spatial packing, fluidity, ion-leak, and integral protein function of membranes in ways that promote substrate transport, ATP production or metabolic efficiency (Murphy 1990, Hulbert and Else 2005, Gerson et al. 2008). Finally, PUFAs may act as signals, either by altering prostaglandin synthesis or directly as "metacrine" hormones (Craig-Schmidt et al. 1987, Forman et al. 1997). For example, n3 and n6 PUFAs are important ligands for peroxisome-proliferator-activated receptors (PPAR) that control numerous genes involved in fatty acid metabolism (Desvergne and Wahli 1999).

PUFA confusion

Exercise performance in birds as well as in other animals can be affected by diet-induced changes in fatty acid unsaturation, or in the ratio of n6 to n3 PUFAs. Unfortunately, the patterns of effects and the interpretation of data differ widely. This has led to a great deal of debate and ample opportunity for future investigation. The two most challenging aspects of studying the effects of fatty acid composition on exercise or any physiological function are: (1) that enriching or depleting particular fatty acids necessarily affects other fatty acids in opposite directions, making interpretation of cause and effect difficult, and (2) that dietary manipulation of fatty acids may simultaneously affect adipose triglycerides, cellular membranes, and signaling pathways, making the isolation of mechanism very challenging.

Some studies suggest that n6 PUFAs enhance exercise performance. Rats fed a high n6 PUFA diet had superior endurance during running on treadmills compared to those fed a high n3 PUFA diet

(Ayre and Hulbert 1997). A similar effect was found for maximum speed of swimming of Atlantic salmon (*Salmo salar*; McKenzie et al. 1998). Ruf et al. (2006) showed that n6 PUFA content of muscle membranes was positively correlated with mass-corrected maximal running speed across a wide variety of mammals, which they suggested could be due to improved function of the Ca^{++} ATPase of the sarcoplasmic reticulum. Pierce et al. (2005) found that maximum metabolic rate during flapping exercise in a flight-wheel respirometer was higher for red-eyed vireos (*Vireo olivaceous*) fed a 58% total unsaturation diet than those fed an 82% total unsaturation diet. However, close inspection of these diets indicates that the 58% diet contained 24% n6 PUFAs while the 82% diet contained only 14% n6 PUFAs. Price and Guglielmo (2009) found that a high dietary ratio of n6 to n3 PUFAs increased maximum metabolic rate during flight-wheel exercise of white-throated sparrows. Moreover, using careful food restriction and refeeding protocols Price and Guglielmo (2009) were able to show that the improved performance was associated with the fatty acid composition of fat stores and not of muscle membranes.

There is also evidence that n3 PUFAs may improve exercise ability. For example, highly aerobic muscles like hummingbird pectoralis have relatively high amounts of n3 PUFAs (Infante et al. 2001). Endurance training in rats and humans is correlated with a decrease in the ratio of n6 to n3 in muscle membranes (Andersson et al. 1998; Helge et al. 1999, 2001), and the n6 to n3 ratio of flight muscle phospholipids decreased during migration in western sandpipers (Guglielmo et al. 2002b). These shifts in n6:n3 with endurance training are intriguing, but could be interpreted either as an adaptive increase in the relative amount of n3 PUFAs to enhance performance, or a depletion of essential n6 PUFAs by training which, according to the studies described earlier, could reduce performance.

Natural doping in shorebirds

During fall migration each year, great numbers of semipalmated sandpipers (*Calidris pusilla*) stopover to refuel on the mudflats of the Bay of Fundy while in transit between breeding areas on the arctic tundra and wintering areas in the West Indies and coasts of Central and South America (Hicklin and Gratto-Trevor 2010). They feed voraciously for a few weeks on *Corophium volutator*, an abundant benthic crustacean, doubling their weight and becoming extremely fat, thereby storing sufficient fuel to complete a non-stop flight of >3200 km over the open ocean to the wintering areas (Hicklin and Gratto-Trevor 2010). Over the course of the stopover the sandpipers also increase the mass of their flight muscles, and the activities of oxidative enzymes (Driedzic et al. 1993). *Corophium*, like many marine invertebrates, is highly enriched (43% of all fatty acids) in n3 PUFAs, such as 20:5n3 and 22:6n3 (Maillet and Weber 2006).

Maillet and Weber (2006, 2007) showed that the high n3 PUFA intake by semipalmated sandpipers causes enrichment of the adipose triglycerides and muscle phospholipids with n3 PUFAs, and that the n3 PUFA content of triglycerides and/or phospholipids was positively correlated with the activities of citrate synthase (CS) and 3-hydroxyacyl-CoA-dehydrogenase (HOAD) in flight muscle, but not of carnitine palmitoyl transferase (CPT). They formulated the natural doping hypothesis, which proposes that high intake of n3 PUFAs increases migratory performance by enhancing the functional capacity of membranes and increasing the aerobic capacity of flight muscles. The n3 PUFAs are thought to activate PPAR-mediated expression of genes that enhance aerobic capacity and the oxidation of fatty acids (Maillet and Weber 2007, Weber 2009). Consistent with this hypothesis, Nagahuedi et al. (2009) showed that sedentary quail (*Colinus virginianus*) can be induced to express high activities of CS, HOAD, CPT, and cytochrome oxidase in flight muscles by daily supplementation with various n3 PUFAs. A retrospective analysis of data on western sandpipers also suggests a link between n3 PUFAs and the oxidative capacity of muscles.

Natural doping in western sandpipers?

In the course of my studies of western sandpipers, I measured flight muscle activities of HOAD, CPT, and CS, and H-FABP concentration (Guglielmo et al. 2002a) as well as fatty acid profiles of plasma lipids (Guglielmo et al. 2002b). To maximize sample size I based plasma fatty acid profiles on total fatty acids present in the combined neutral lipids (NL) and phospholipids (PL) because non-esterified fatty acids were not measured in all samples and their concentrations were relatively low (Guglielmo et al. 2002b). The NL and PL therefore mostly determine the external milieu of fatty acids to which muscle cells are exposed. To eliminate spurious correlations I tested for significant correlation within migrants and non-migrants as well as across seasons, and used analysis of covariance (ANCOVA) with enzyme activity (or H-FABP) as a dependent variable, migratory state as a class variable, and fatty acid mass percent as a covariate. Total unsaturation was

Table 1 Pearson correlations (r) between pectoralis muscle variables and combined plasma neutral lipids and phospholipids of western sandpipers

Muscle variable	Total FA	UNSAT	PUFA	18:0	20:5n3	22:6n3
All birds ($n=23$)						
HOAD	0.58**	0.64**	0.70***	−0.50*	0.74***	0.62**
CPT	0.36	0.62**	0.64**	−0.56*	0.74***	0.67***
CS	0.35	0.37	0.31	−0.45*	0.42*	0.39
H-FABP	0.63**	0.48*	0.24	−0.51*	0.49*	0.37
Migrants ($n=12$)						
HOAD	0.52	0.69*	0.68*	−0.38	0.76**	0.56
CPT	−0.12	0.63*	0.67*	−0.56	0.64*	0.72*
CS	0.41	0.42	0.42	−0.37	0.44	0.40
H-FABP	0.06	0.03	−0.07	−0.13	0.07	0.09
Non-migrants ($n=11$)						
HOAD	−0.01	0.32	0.43	−0.23	0.32	0.38
CPT	0.37	0.46	0.47	−0.44	0.51	0.65*
CS	−0.06	−0.13	−0.10	−0.37	0.15	0.20
H-FABP	0.25	0.11	−0.07	−0.11	0.20	0.15

*$P<0.05$, **$P<0.005$, ***$P<0.0005$.
HOAD, CPT, CS, activities (μmol/min mg/protein) of 3-hydroxyacyl-CoA-dehydrogenase, carnitine palmitoyl transferase, citrate synthase; H-FABP, % cytosolic protein of heart-type fatty acid binding protein; Total FA, concentration (nmol/ml) of fatty acids; and UNSAT, PUFA, 18:0, 20:5n3 and 22:6n3, mass percent of all unsaturates, polyunsaturates, 18:0, 20:5n3, and 22:6n3.

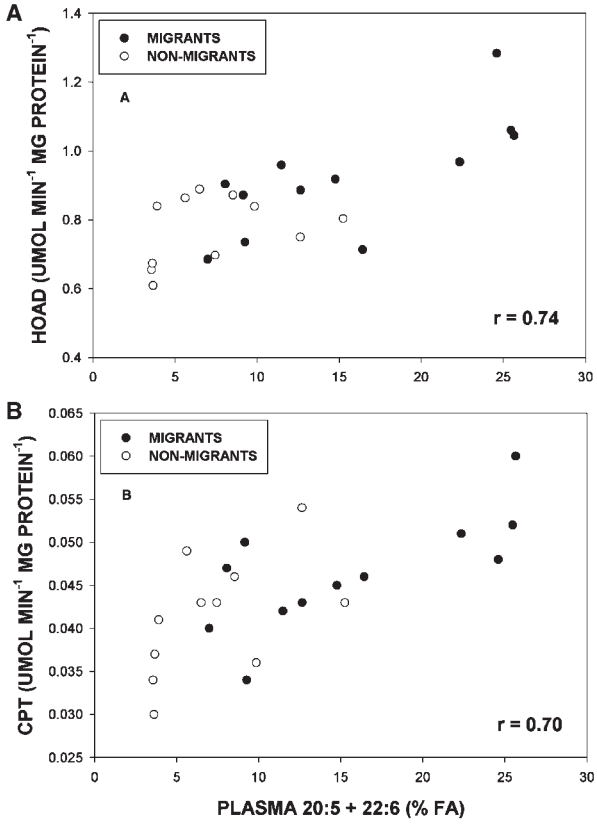

Fig. 2 The activities of (A) HOAD, and (B) CPT from pectoralis muscle plotted against the combined mass percent of eicosapentanoic acid (20:5n3) and docosahexanoic acid (22:6n3) in the plasma neutral lipids and phospholipids of non-migratory (winter resident in Panama) and migratory (refueling in British Columbia, Canada) western sandpipers. The Pearson correlation coefficient (r) is shown for all data combined ($P<0.001$). ANCOVA indicated that there was no effect of migratory state on either the slopes or intercepts of the relationships (see text).

calculated as the sum of all monounsaturated and PUFAs. Relationships were slightly stronger when fatty acid profile was expressed as mass percent than as plasma concentration, and so I present the results using mass percent.

Activities of HOAD and CPT were positively related with unsaturation and polyunsaturation of plasma NL and PL across seasons and within migrants (Table 1). CS activity and H-FABP were not significantly related to unsaturation or to polyunsaturation. Mass percentages of individual fatty acids (16:0, 16:1n7, 18:0, 18:1n9, 18:2n6, 18:3n3, 20:4n6, 20:5n3, and 22:6n3) were tested to determine if any were specifically correlated with activities of enzymes or levels of H-FABP. The PUFAs 20:5n3 and 22:6n3 were the only fatty acids that were significantly and consistently positively related with HOAD and CPT activities across seasons and within migrants alone. For simplicity, I used summed 20:5n3 and 22:6n3 mass percent as the covariate in ANCOVA (Fig. 2). HOAD and CPT activities increased with increasing 20:5n3 + 22:6n3 ($P<0.02$), and there was no difference in slopes ($P>0.41$) or intercepts ($P>0.71$) between migrants and non-migrants for either enzyme. These relationships were not evident for CS or H-FABP. Muscle enzymes and H-FABP were negatively correlated with 18:0 mass percent across, but not within seasons.

Even with this relatively small sample of birds, western sandpipers clearly show a relationship at the individual level and, regardless of season, between plasma n3 PUFA content and CPT and HOAD activities, two enzymes known to have PPAR-activated transcription (Desvergne and Wahli 1999). This is consistent with the natural doping hypothesis, but the dietary or endogenous regulatory mechanisms underlying it are unknown.

Can natural doping work?

Although the idea of birds priming their flight muscles for migration by natural doping with high n3 PUFA foods is intriguing, it is not without

difficulties. In the case of the semipalmated sandpipers at the Bay of Fundy, fatness of the body, fatty acid composition, muscle biochemistry and possibly other factors all change over the course of a long stopover, making it difficult to definitively link n3 PUFAs with the oxidative metabolism of muscles by using correlation alone. On the other hand, controlled experiments with quail (Nagahuedi et al. 2009), and the relationships seen in western sandpipers, which were wintering or making brief stopovers, do indicate that this link exists. It is unlikely that most migratory bird species, particularly passerine landbirds, have access to high n3 PUFA foods, such as marine invertebrates. Their fat stores are usually low in n3 PUFAs, and it is more usual to observe significant amounts of 18:2n6 (Pierce and McWilliams 2005, Klaiman et al. 2009). Klaiman et al. (2009) showed that muscle phospholipids and fat-depot triglycerides of white-throated sparrows become highly enriched in 18:2n6 during migration, with no change in n3 PUFAs. Yet these birds were also shown to greatly increase muscle FAT/CD36, FABPpm, H-FABP, CS, CPT and HOAD (McFarlan et al. 2009). Similar changes in muscle transporters and enzymes were recently observed in response to photoperiodic changes alone in white throated sparrows fed a constant diet (Zajac D, Cerasale D, Guglielmo CG, unpublished data). These results indicate that n3 PUFAs are not necessary for the suite of changes that occur in the oxidative fuel metabolism of flight muscles, and that other endogenous mechanisms are probably more important.

It has also not been demonstrated that enrichment of fat stores and membranes with n3 PUFAs, and the resulting increases in the activities of enzymes, actually improve exercise performance or are necessary for endurance flight. Sandpipers that refuel at the Bay of Fundy have already flown hundreds or thousands of kilometers from breeding areas to the north and west (Hicklin and Gratto-Trevor 2010). The transformation of fatty acid composition that occurs during stopover indicates that they did not have access to high n3 PUFA foods on the breeding areas, yet they were able to successfully migrate. The balance of the evidence indicates that maximal performance of birds, mammals and fish is improved when they are enriched with n6 PUFAs. It may be that metabolic efficiency, which would be favored for migratory flight, could be enhanced differently. McWilliams and Pierce (2006) reported that European starlings (*Sturnus vulgaris*) expended 13% less energy over a 6-h flight in a wind tunnel when fed a highly polyunsaturated diet (enriched mostly with 18:2n6) compared to a monounsaturated diet. It is clear that controlled experiments and field studies are desperately needed to clarify cause and effect, and to determine if unsaturation alone, or a simple difference in the placement of double bonds (n3 or n6) have any biologically meaningful effects on migration in birds.

Are migratory bats like flying birds or like running mammals?

Studying migratory bats may allow us to test the generality of the bird model for the evolution of migration by flight in vertebrates, as well as simply provide a greater knowledge of these elusive animals. Bats evolved flight independently of birds, but they are subject to many of the same selection pressures for flight and migration. Unfortunately, the physiology of migratory bats is so poorly known that most discussion must be based on hypotheses and speculation. McGuire and Guglielmo (2009) reviewed the available information on the physiology of bat migration and compiled a list of future directions for research. I will emphasize some key predicted similarities to birds as well as features of bats that may be unique.

One area of evolutionary convergence with birds should be the capacity for migratory bats to fuel flight with exogenous fatty acids. The power requirements for flight are similar between birds and bats (Winter and von Helverson 1998), and are much greater than the aerobic capacity of cursorial mammals. Indirect calorimetry of flying bats indicates that they can support flight by the oxidation of fatty acids (Welch et al. 2008), but it has not revealed whether the source of the fatty acids is intramyocyte fat droplets or extramuscular adipose stores which should require high levels of fatty acid transporters (FAT/CD36, FABPpm and H-FABP) in flight muscles. It will be interesting to discover whether bats follow the classic mammalian exercise model or if they are similar to birds. Many other basic questions remain unanswered or very poorly documented, such as: (1) do migrating bats store large fat loads? (2) what is the mixture of fat and protein catabolized during migratory flight? (3) do bats fast while flying, feed on the wing, or use food ingested pre-flight? (4) does the fatty acid composition of fat stores or muscle membranes change during migration? and (5) does the aerobic capacity of muscle increase (oxidative enzymes, capillarity, mitochondrial volume) during migration?

Bats may also differ from birds in very interesting ways that would influence their physiology and

behavior during migration (McGuire and Guglielmo 2009). For example, bats could use torpor on a facultative and flexible basis to save energy, and there is evidence that hoary bats (*Lasiurus cinereus*) do so during migration (Cryan and Wolf 2003). Migrating hummingbirds are known to use torpor during stopover (Carpenter and Hixon 1988, Hiebert 1993), and there is some evidence for other birds using hypothermia during migration (Butler and Woakes 2001, Wojciechowski and Pinshow 2009). However, most birds cannot use torpor and may expend up to 70% of their energy during migration at stopover rather than in flight (Wikelski et al. 2003). The regular use of torpor between flights by bats could greatly increase their energy efficiency and overall migration speed. Another major difference between birds and bats is that bats may not completely segregate reproductive and migratory life stages. In spring, female bats may migrate while pregnant which may affect flight costs and food requirements, and limit their ability to use torpor (Cryan and Wolf 2003). Similarly, mating may occur during fall migration (Cryan 2008), and so may influence the budgets of time and energy of bats seeking mating opportunities.

There is a pressing need to learn more about the behavior, ecology and physiology of migration in bats, particularly in light of recent concerns about high mortalities at wind-energy developments (Kunz et al. 2007). We are currently studying hoary bats (*Lasiurus cinereus*) and silver-haired bats (*Lasionycteris noctivagans)* to answer study some of the questions posed above (McGuire LP, Fenton MB, Guglielmo CG, unpublished data); however, bat migration deserves much more attention by a wider community of researchers.

The advanced facility for avian research

Fully understanding the physiology of migratory birds and bats will not be possible without specialized facilities to keep captives in excellent health and for use in conducting experiments. With support from the Canada Foundation for Innovation and the Ontario Research Fund, the University of Western Ontario recently constructed the Advanced Facility for Avian Research (AFAR; http://www.birds.uwo.ca). The AFAR has specialized indoor and outdoor holding areas for a wide variety of bird species, including shorebirds. Long-distance migration can be studied using a hypobaric climatic wind tunnel in which barometric pressure, wind speed, humidity and temperature can be independently controlled to simulate altitudes of up to 7000 m. A quantitative magnetic resonance (QMR) body-composition analyzer is available to quickly and non-invasively measure fat, lean mass and water mass both pre-flight and post-flight with accuracies of ±10, ±2, and ±2%, respectively (Taicher et al. 2003, Guglielmo CG, Gerson AR, McGuire LP, Seewagen CL, unpublished data). Recent experiments using QMR showed that, as theory predicts, water-stressed birds catabolize more lean mass at rest than do control birds (Gerson AR. Guglielmo CG, unpublished data); the next step will be to test birds flying under different conditions of humidity. Over the coming years the AFAR will allow researchers to answer similar long-standing questions about energetics, fuel use, water balance, respiration and other aspects of migration physiology.

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